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CLINICAL RESEARCH

Short-Term Hemodynamic Effects of Apelin in Patients With Pulmonary Arterial Hypertension

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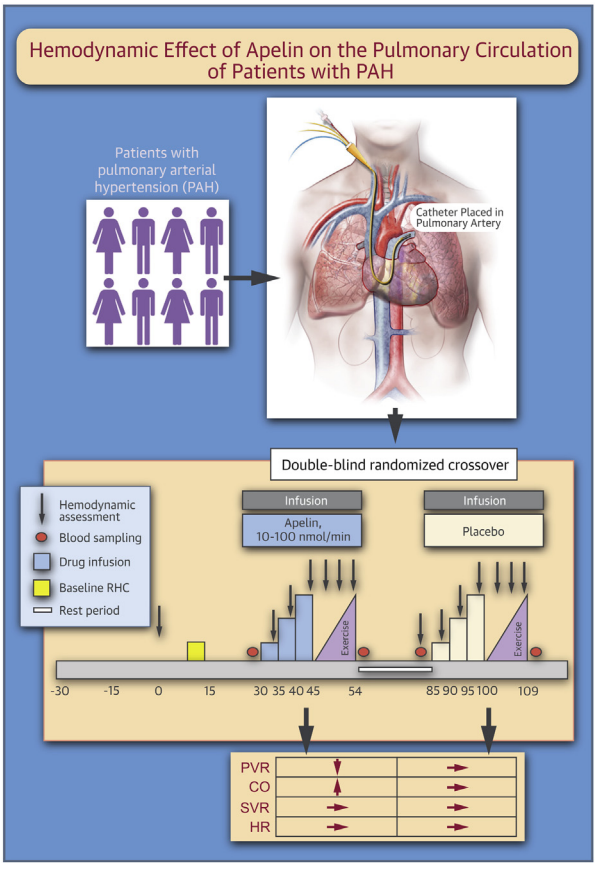
VISUAL ABSTRACT



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HIGHLIGHTS

- **The effects of apelin on pulmonary hemodynamics in patients with PAH are unknown.**
- **Systemic infusion caused a significant reduction in pulmonary vascular resistance and increase in cardiac output without a change in heart rate or systemic vascular resistance.**
- **This effect was most prominent in the subgroup of patients receiving concomitant PDE5 inhibition.**
- **Apelin agonism is a novel potential therapeutic target for PAH.**

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SUMMARY

Apelin agonism causes systemic vasodilatation and increased cardiac contractility in humans, and improves pulmonary arterial hypertension (PAH) in animal models. Here, the authors examined the short-term pulmonary hemodynamic effects of systemic apelin infusion in patients with PAH. In a double-blind randomized crossover study, 19 patients with PAH received intravenous (Pyr[1])apelin-13 and matched saline placebo during invasive right heart catheterization. (Pyr[1])apelin-13 infusion caused a reduction in pulmonary vascular resistance and increased cardiac output. This effect was accentuated in the subgroup of patients receiving concomitant phosphodiesterase type 5 inhibition. Apelin agonism is a novel potential therapeutic target for PAH.
(Effects of Apelin on the Lung Circulation in Pulmonary Hypertension; NCT01457170)

ABBREVIATIONS AND ACRONYMS

CO = cardiac output
FA = formic acid
NO = nitric oxide
PAEC = pulmonary artery endothelial cells
PAH = pulmonary arterial hypertension
PDE5 = phosphodiesterase-5
PVR = pulmonary vascular resistance
SVR = systemic vascular resistance

Pulmonary arterial hypertension (PAH) is a disease in which functional and structural changes of the pulmonary vasculature cause a progressive increase in pulmonary vascular resistance (PVR), leading to pressure overload of the right ventricle and premature death. This is thought to arise from an imbalance between vasodilator and vasoconstrictor mediators. In addition, structural changes of the vessel wall (remodeling) occur due to proliferation of the endothelium, vascular smooth muscle cells, and fibroblasts leading to progressive obstruction of the pulmonary vascular bed (1). Although there are current therapies in PAH targeting the nitric oxide (NO)-cyclic GMP, endothelin, and prostacyclin pathways, there remains an unacceptably low 3-year survival of 63% (2). Further novel therapeutic interventions are therefore needed to improve the outlook in this progressive fatal condition.

Apelin is an endogenous peptide that was first discovered in 1998. It binds to a previously orphaned G-protein-coupled receptor, now termed the apelin receptor (3). Apelin receptors are present on endothelial cells, vascular smooth muscle cells, and cardiomyocytes (4). In preclinical models, apelin signaling exerts major effects on both vascular tone and cardiac contractility leading to vasorelaxation (5), falls in arterial blood pressure and systemic venous tone (6–9), and potent inotropism (10) with increased cardiac contractility.

We have previously conducted clinical studies looking at the effects of apelin in healthy volunteers and patients with heart failure (11,12). We showed apelin to be a direct coronary and peripheral vasodilator, to increase myocardial contractility, and to reduce peak and end-diastolic left ventricular pressure (13). We also demonstrated that these inotropic actions were maintained during prolonged 6-h infusions (12).

Apelin infusion improves pulmonary vascular hemodynamics in 2 animal models of PAH (14,15), and this may translate into benefit for patients with PAH by reducing PVR and increasing cardiac output (CO). Given these preclinical and clinical data, we sought to determine the short-term pulmonary hemodynamic effects of intravenous apelin infusion in patients with PAH.

METHODS

This was a double-blind randomized crossover study of the short-term hemodynamic effects of apelin in

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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patients with PAH. All studies were performed with written informed consent of the participants, with the approval of the local ethics committee, and in accordance with the Declaration of Helsinki.

SUBJECTS. Patients with PAH were recruited from the Golden Jubilee National Hospital in Glasgow and Hammersmith Hospital in London, both of which are specialist national centers for PAH. Patients were either new patients attending for diagnostic assessment or patients with known PAH who have been receiving stable doses of approved mono- or combination PAH therapy for at least 2 months before study. Inclusion criteria were: 1) PAH that is idiopathic, associated with drugs/toxins, associated with connective tissue disease, or heritable; 2) mean pulmonary arterial pressure ≥25 mm Hg, pulmonary capillary wedge pressure ≤15 mm Hg with a normal or reduced CO; and 3) stable World Health Organization functional capacity of grade II to IV for 3 months before study. PAH patients were excluded if they had significant left ventricular dysfunction, chronic lung disease (FEV_1/FVC <60%; abnormal lungs on computed tomography), or chronic thromboembolic pulmonary hypertension. Further exclusion criteria included bleeding diathesis, women of childbearing potential, systolic blood pressure >190 mm Hg or <100 mm Hg, malignant arrhythmias, renal or hepatic failure, or hemodynamically significant valvular heart disease.

All subjects fasted for at least 4 h before the procedure and avoided alcohol or caffeine for 24 h before the study. Patients delayed taking routine medication on the morning of the right heart catheterization until completion of the study protocol.

DRUGS. The effects of apelin agonism were determined using synthetic-grade (Pyr^1)apelin-13 (Clinalfa, Laufelfingen, Switzerland) (11,13). This was administered after dissolution in 0.9% physiological saline (Baxter, Deerfield, Illinois) under aseptic conditions on the study day.

PLASMA APELIN CONCENTRATIONS. In a group of patients (n = 8), plasma apelin concentrations were measured to ensure that the infusion had raised apelin levels. Plasma (Pyr^1)apelin-13 concentration was determined using immunoprecipitation followed by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) analysis. Briefly, human plasma (500 μl, kept on ice) was first treated with 2 × 0.75 ml cold 0.1% formic acid (FA) in isopropanol. Following centrifugation, the supernatants were dried under nitrogen then reconstituted in phosphate buffered saline/Tween. For immunoprecipitation, an anti-(pyr^1)apelin-13 monoclonal antibody (Bristol-Myers Squibb, Princeton, New Jersey) was bound to high capacity Protein G magnetic beads (Promega, Madison, Wisconsin). The reconstituted human plasma was incubated overnight at room temperature with 25 μl of the antibody-bead slurry. After multiple washes, (pyr^1)apelin-13 was eluted from the bead complex using 0.1% bovine serum albumin/0.1% FA in 90:10 water to methanol. The eluates were analyzed by LC/MS/MS on a Sciex API6500 mass spectrometer (Sciex, Redwood City, California) coupled to a Shimadzu Nexera X2 LC-30AD pump and Sil-30AC MP autosampler (both Shimadzu, Kyoto, Japan). Gradient elution was performed on a Waters BEH300 2.1 × 50 mm column (Waters, Milford, Massachusetts) using a mobile phase of 0.1% FA and 0.1% FA in acetonitrile. The transitions monitored were m/z 384.2→424.8 for (pyr^1)apelin-13 and m/z 387.4→426.8 for the internal standard (^{13}C,^{15}N-(pyr^1)apelin-13 from Innovagen [Lund, Sweden]). For normalization, the internal standard was added to all human plasma and calibration curve samples before starting the sample extraction protocol. Calibration curves were generated by spiking (pyr^1)apelin-13 standard into blank human plasma (purchased from Bioreclamation [Westbury, New York] and heated at 37°C overnight to remove any endogenous apelin) and extracting apelin following the identical sample extraction protocol described above. The concentration of (pyr^1)apelin-13 in clinical human plasma was calculated on the basis of the calibration curves, using the peak area ratio of measured apelin to internal standard. The limit of quantification of the assay was approximately 4 pmol/l.

PULMONARY HEMODYNAMIC ASSESSMENT. Resting hemodynamic evaluation was performed as per routine protocol and included right atrial pressure, right ventricular pressure, pulmonary arterial pressure, and pulmonary arterial wedge pressure.

Cardiac output (CO) was measured by either thermodilution (Golden Jubilee National Hospital) or the Fick principle (Hammersmith Hospital). Thermodilution CO was taken from the mean of at least 3 values that differed by <10%. The Fick method determined CO by arterial blood sampling and sampling of mixed venous blood from the pulmonary artery via the Swan-Ganz catheter. Oxygen consumption was measured using the Fitmate (Cosmed, Rome, Italy). CO was then calculated using the modified Fick equation: CO = O_2 consumption/(O_2 content of arterial blood − O_2 content of mixed venous blood). PVR was calculated by dividing the difference between mean pulmonary artery and pulmonary arterial wedge pressures by the CO.

PROTOCOL. Each study was conducted in a quiet and temperature-controlled investigation suite with subjects in the supine position. Heart rate, electrocardiogram, pulse oximetry, and systemic arterial blood pressure were monitored continuously throughout.

An 8-F sheath was placed in the right internal jugular vein using ultrasound imaging, or brachial vein using standard aseptic techniques. A Swan-Ganz catheter (7-F, 2 lumen, thermodilution pressure-measuring tipped catheter; Edwards Lifesciences, Irvine, California) was passed into the pulmonary artery.

In a randomized, double-blinded, crossover design, each participant received three 5-min intravenous infusions of ascending doses of (Pyr^1) apelin-13 at 10, 30, and 100 nmol/min (11,13) or matched saline placebo. The order of (Pyr^1)apelin-13 or saline placebo was randomized. Hemodynamic data were collected at the end of each 5-min infusion period. The apelin and saline placebo infusions were separated by a 30-min washout period before commencing the second infusion period. A 30-min washout was chosen on the basis of our previous clinical studies that showed that apelin was rapidly cleared from the circulation with a short plasma half-life of no longer than 8 min (11).

EXERCISE TESTING PROTOCOL. In the days before right heart catheterization, subjects underwent a progressive incremental exercise test on an ergometer to determine the maximal exercise capacity, starting at 0 W for 3 min and adding 10 W every minute until the symptom-limited maximum was reached. This was used to calculate exercise work rates for individuals during the exercise component of the study. On the day of study and at the end of each 15-min infusion period (100 nmol/min of (Pyr^1)apelin-13 or matched saline placebo), participants underwent an exercise protocol. This exercise study was carried out in the supine position with a cycle ergometer secured to the catheterization table (Golden Jubilee National Hospital) or on an echocardiogram exercise stress table (Cranlea Human Performance, United Kingdom). The patient was instructed to pedal at a rate of 60 rpm. Hemodynamic measurements were taken at rest, during unloaded exercise and during loaded exercise (20% and 40% of the previously determined maximal exercise capacity during their progressive incremental exercise test).

DATA AND STATISTICAL ANALYSIS. The primary endpoint of the study was the change in PVR from baseline. This is a standard and well-validated primary or secondary endpoint in clinical trials for PAH therapies. Secondary outcomes included change in CO and systemic vascular resistance (SVR). On the basis of previous studies (16–21) investigating the short-term pulmonary hemodynamic effects of PAH treatments, we calculated that to see an effect size of 20% (SD of 2.13 WU/149 dyne·s·cm^{-5}), we would need a sample size of around 21 (21) at 80% power and 2-sided $p < 0.05$. Data are reported as mean ± SEM and analyzed with repeated-measures analysis of variance with post hoc Bonferroni corrections (GraphPad Prism 7 software, GraphPad Software, San Diego, California). Statistical significance was taken as 2-sided $p < 0.05$.

TABLE 1 Demographic and Hemodynamic Characteristics at Baseline

Age, yrs	51.8 ± 3.2
Sex	
Male	7/19 (37)
Female	12/19 (63)
Diagnosis	
IPAH	17/19 (89)
CTD PH	2/19 (11)
Smoker	
Never	11/19 (58)
Current	4/19 (21)
Ex	4/19 (21)
Hb, g/dl	14.1 ± 0.4
BMI, kg/m^2	29.1 ± 1.1
Medication	
PDE5i	11/19 (58)
ERA	9/19 (47)
Prostacycline	3/19 (16)
Treatment naive	5/19 (26)
Baseline hemodynamics	
PVR, WU	9.1 ± 0.9
CO, l/min	4.8 ± 0.3
Heart rate, beats/min	72 ± 2.9
mPAP, mm Hg	48.1 ± 2.7
SV, ml	69.8 ± 4.9
MAP, mm Hg	87.8 ± 2.6
RAP, mm Hg	6.1 ± 0.8
PAWP, mm Hg	7.6 ± 0.7
SVR, dyn	1,413 ± 74.1

Values are mean ± SEM or n/N (%).

BMI = body mass index; CO = cardiac output; CTD PH = connective tissue disease pulmonary hypertension; ERA = endothelin receptor antagonist; Hb = hemoglobin; IPAH = idiopathic pulmonary arterial hypertension; MAP = mean arterial pressure; mPAP = mean pulmonary artery pressure; NYHA = New York Heart Association; PAWP = pulmonary arterial wedge pressure; PDE5i = phosphodiesterase inhibitor; PVR = pulmonary vascular resistance; RAP = right atrial pressure; SV = stroke volume; SVR = systemic vascular resistance.

RESULTS

Study participants were predominantly female and middle-aged (Table 1). All studies were well tolerated with no serious adverse events or electrocardiographic changes during apelin administration. Nineteen subjects were included in the analysis (2 subjects were excluded because of protocol deviations). Before apelin infusion, there were no detectable basal plasma apelin concentrations in patients with PAH. Following infusion with (Pyr^1)apelin-13, there was a marked increase in plasma apelin concentrations (peak concentration, 10,681 ± 4,184 pmol/l). The individual data for each study participant are included in Table 2.

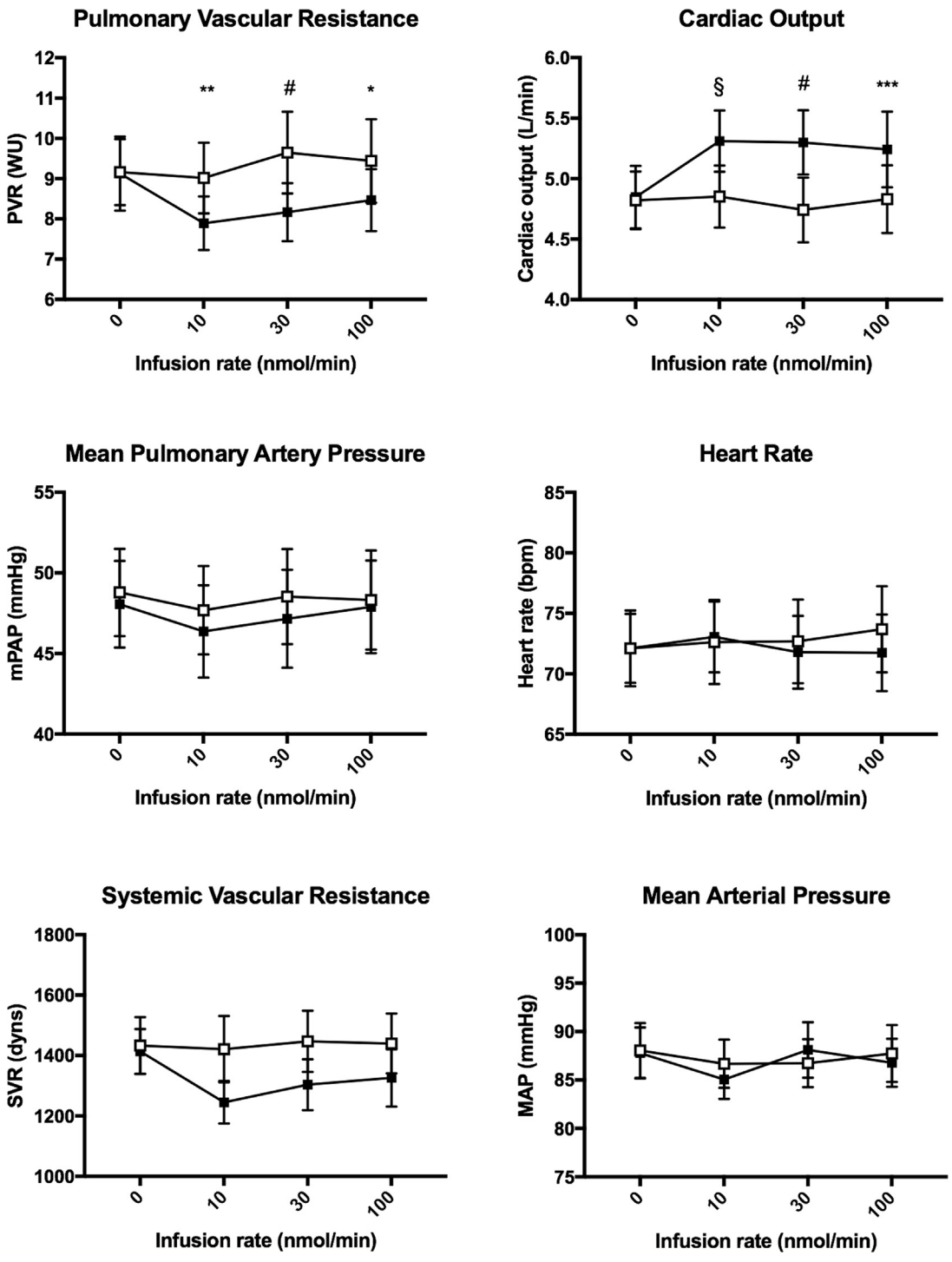
TABLE 2 Hemodynamic Data

	Apelin, nmol/min				Saline Placebo, min			
	Baseline	10	30	100	Baseline	5	10	15
All subjects (n = 19)								
PVR, WU	9.1 ± 0.9	7.9 ± 0.7	8.2 ± 0.7	8.5 ± 0.8	9.2 ± 0.8	9.0 ± 0.9	9.6 ± 1.0	9.4 ± 1.0
CO, l/min	4.8 ± 0.3	5.3 ± 0.3	5.3 ± 0.3	5.2 ± 0.3	4.8 ± 0.2	4.9 ± 0.3	4.7 ± 0.3	4.8 ± 0.3
HR, beats/min	72 ± 2.9	73 ± 2.9	72 ± 3.0	72 ± 3.2	72 ± 3.1	73 ± 3.5	73 ± 3.5	74 ± 3.6
mPAP, mm Hg	48 ± 2.7	46 ± 2.9	47 ± 3.0	48 ± 2.9	49 ± 2.7	48 ± 2.7	49 ± 3.0	48 ± 3.1
SV, ml	70 ± 4.9	75 ± 4.8	77 ± 4.8	77 ± 5.7	70 ± 4.8	70 ± 4.8	69 ± 5.0	69 ± 5.3
MAP, mm Hg	88 ± 2.6	85 ± 2.0	88 ± 2.9	87 ± 2.5	88 ± 2.8	87 ± 2.5	87 ± 2.5	88 ± 2.9
SVR, dyne	1,413 ± 74	1,245 ± 70	1,303 ± 84	1,326 ± 95	1,433 ± 94	1,421 ± 110	1,447 ± 101	1,440 ± 99
Treatment naive (n = 5)								
PVR, WU	7.4 ± 1.2	6.6 ± 01.0	7.2 ± 1.3	7.2 ± 1.3	8.1 ± 1.0	7.4 ± 1.3	7.4 ± 1.4	7.2 ± 1.4
CO, l/min	5.1 ± 0.4	5.3 ± 0.5	5.2 ± 0.6	5.6 ± 0.7	5.0 ± 0.5	5.1 ± 0.6	5.1 ± 0.6	5.3 ± 0.7
HR, beats/min	64 ± 4.7	62 ± 5.5	61 ± 4.5	64 ± 5.6	64 ± 6.5	66 ± 6.8	65 ± 5.6	66 ± 5.8
mPAP, mm Hg	43 ± 2.7	41 ± 3.1	42 ± 4.3	43 ± 3.6	45 ± 2.4	42 ± 4.0	41 ± 3.6	40 ± 4.0
SV, ml	81 ± 9.3	88 ± 10.1	86 ± 9.5	88 ± 11.2	80 ± 9.0	79 ± 8.0	80 ± 9.5	82 ± 12.3
MAP, mm Hg	85 ± 2.7	81 ± 3.1	88 ± 2.9	87 ± 4.5	85 ± 3.2	84 ± 3.7	84 ± 5.4	81 ± 3.5
SVR, dyne	1,280 ± 103	1,201 ± 182	1,354 ± 226	1,283 ± 283	1,365 ± 227	1,329 ± 244	1,363 ± 294	1,266 ± 240
On treatment (n = 14)								
PVR, WU	9.7 ± 1.2	8.4 ± 0.8	8.5 ± 0.9	8.9 ± 0.9	9.6 ± 1.1	9.6 ± 1.1	10.4 ± 1.2	10.2 ± 1.3
CO, l/min	4.8 ± 0.3	5.3 ± 0.3	5.3 ± 0.3	5.1 ± 0.3	4.8 ± 0.3	4.8 ± 0.3	4.6 ± 0.3	4.7 ± 0.3
HR, beats/min	75 ± 3.3	77 ± 2.9	76 ± 3.3	75 ± 3.6	75 ± 3.4	75 ± 4.0	76 ± 4.1	77 ± 4.2
mPAP, mm Hg	50 ± 3.4	48 ± 3.6	49 ± 3.8	50 ± 3.6	50 ± 3.6	50 ± 3.3	51 ± 3.6	51 ± 3.7
SV, ml	66 ± 5.6	71 ± 5.1	73 ± 5.5	72 ± 6.5	66 ± 5.6	67 ± 5.8	65 ± 5.6	65 ± 5.5
MAP, mm Hg	89 ± 3.5	86 ± 2.5	88 ± 3.8	87 ± 3.0	89 ± 3.7	88 ± 3.1	88 ± 2.8	90 ± 3.6
SVR, dyne	1,461 ± 92	1,261 ± 74	1,285 ± 87	1,339 ± 99	1,457 ± 104	1,454 ± 125	1,477 ± 97	1,502 ± 104
On PDE5 inhibitors (n = 11)								
PVR, WU	10.9 ± 1.2	9.1 ± 0.9	9.2 ± 1.0	10.8 ± 1.0	10.6 ± 1.1	10.6 ± 1.2	11.6 ± 1.3	11.4 ± 1.4
CO, l/min	4.5 ± 0.3	5.1 ± 0.3	5.1 ± 0.4	4.9 ± 0.4	4.5 ± 0.3	4.5 ± 0.3	4.3 ± 0.3	4.4 ± 0.3
HR, beats/min	75 ± 3.8	78 ± 3.3	76 ± 3.7	76 ± 4.3	75 ± 4.0	75 ± 4.7	76 ± 4.8	77 ± 5.0
mPAP, mm Hg	52 ± 3.9	51 ± 3.9	52 ± 4.2	52 ± 4.0	53 ± 3.7	52 ± 3.5	54 ± 3.7	54 ± 3.9
SV, ml	62 ± 6.7	68 ± 6.2	71 ± 6.8	69. ± 8.0	63 ± 6.8	64 ± 7.0	61 ± 6.8	62 ± 6.6
MAP, mm Hg	85 ± 3.0	85 ± 2.3	85 ± 2.2	85 ± 2.2	87 ± 2.7	86 ± 2.1	86 ± 2.8	87 ± 2.5
SVR, dyne	1,494 ± 114	1,289 ± 86	1,290 ± 92	1,382 ± 108	1,495 ± 116	1,513 ± 149	1,543 ± 112	1,542 ± 120
On treatment without PDE5 inhibitors (n = 3)								
PVR, WU	5.3 ± 1.3	5.6 ± 1.4	5.9 ± 1.4	5.6 ± 1.4	5.6 ± 1.5	5.9 ± 1.4	6.2 ± 1.6	5.9 ± 1.4
CO, l/min	5.8 ± 0.6	6.0 ± 0.7	6.1 ± 0.4	5.9 ± 0.5	5.6 ± 0.2	5.7 ± 0.1	5.6 ± 0.3	5.7 ± 0.3
HR, beats/min	73 ± 7.7	72 ± 6.8	73 ± 7.8	70 ± 6.8	74 ± 7.9	74 ± 8.5	74 ± 8.2	76 ± 9.1
mPAP, mm Hg	41. ± 5.9	39 ± 7.4	40 ± 8.0	41 ± 8.2	40 ± 8.5	41 ± 8.1	42 ± 9.5	42 ± 8.7
SV, ml	80 ± 0.6	83 ± 1.7	84 ± 3.5	85 ± 3.4	77 ± 5.3	79 ± 6.9	77 ± 3.7	76 ± 5.1
MAP, mm Hg	103 ± 8.4	92 ± 8.2	101 ± 15.6	91 ± 12.9	98 ± 15.2	95 ± 13.8	93 ± 9.0	102 ± 14.2
SVR, dyne	1,342 ± 109	1,157 ± 160	1,267 ± 276	1,178 ± 257	1,318 ± 256	1,238 ± 195	1,238 ± 149	1,357 ± 233
Treatment naive and treatment without PDE5 inhibitors (n = 8)								
PVR, WU	6.7 ± 0.9	6.2 ± 0.8	6.7 ± 0.9	6.6 ± 1.0	7.2 ± 0.9	6.8 ± 0.9	7.0 ± 1.0	6.7 ± 1.0
CO, l/min	5.4 ± 0.3	5.6 ± 0.4	5.6 ± 0.4	5.7 ± 0.5	5.2 ± 0.3	5.3 ± 0.4	5.3 ± 0.4	5.4 ± 0.5
HR, beats/min	68 ± 4.1	66 ± 4.4	66 ± 4.3	66 ± 4.1	68 ± 4.9	69 ± 5.1	68 ± 4.6	70 ± 5.0
mPAP, mm Hg	42 ± 2.5	40 ± 3.1	41 ± 3.7	42 ± 3.5	43 ± 3.2	42 ± 3.6	42 ± 3.8	41 ± 3.7
SV, ml	81 ± 5.6	86 ± 6.1	85 ± 5.8	87 ± 6.8	79 ± 5.7	79 ± 5.3	79 ± 5.8	80 ± 7.6
MAP, mm Hg	92 ± 4.6	85 ± 3.8	93 ± 5.9	89 ± 5.5	90 ± 5.8	88 ± 5.4	88 ± 4.7	89 ± 6.3
SVR, dyne	1,303 ± 72	1,185 ± 121	1,322 ± 164	1,238 ± 181	1,348 ± 160	1,295 ± 160	1,316 ± 184	1,300 ± 163

Values are mean ± SEM.

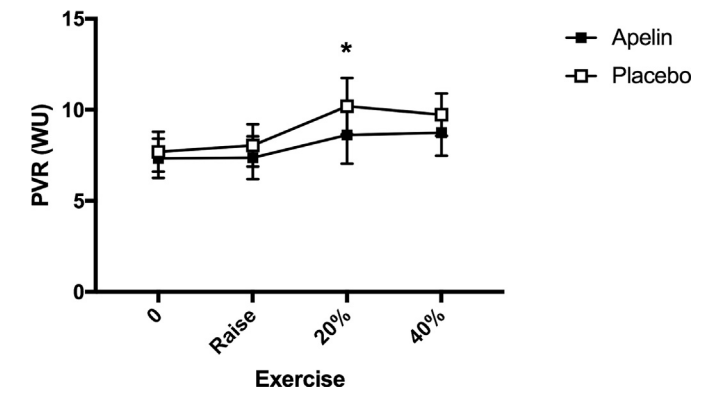
HR = heart rate; other abbreviations as in Table 1.

FIGURE 1 Hemodynamic Changes During Apelin Infusion in Patients With PAH



Hemodynamic changes during infusion of (Pyr[1])apelin-13 **(solid squares)** or matched saline placebo **(open squares)** in patients with pulmonary arterial hypertension (PAH) in pulmonary vascular resistance (PVR), cardiac output, mean pulmonary artery pressure (mPAP), heart rate, systemic vascular resistance (SVR), and mean arterial pressure (MAP). Data are reported as mean ± SEM. *$p = 0.0089$, **$p = 0.002$, ***$p < 0.0006$, §$p = 0.0001$, and #$p \leq 0.0001$ 2-way analysis of variance with post hoc Bonferroni tests. bpm = beats per minute.

FIGURE 2 Effect of Apelin and Exercise on PVR



Pulmonary vascular resistance during infusion of (Pyr[1])apelin-13 or matched saline placebo in patients with pulmonary arterial hypertension while undergoing exercise protocol. A zero reflects resting measurements, raise reflects measurements taken with legs raised in ergometers pedals at rest, then further measurements at 20% and 40% of previous erect maximal cardiopulmonary exercise test performance. Data are reported as mean ± SEM. *p = 0.019, 2-way analysis of variance with post hoc Bonferroni tests. PVR = pulmonary vascular resistance.

HEMODYNAMIC OUTCOMES. No time-order effects were observed. In comparison to saline placebo, (Pyr[1])apelin-13 infusion reduced PVR ($p < 0.0001$) with an effect that appeared maximal at 30 nmol/min (Figure 1). (Pyr[1])apelin-13 infusion increased CO (Figure 1) ($p < 0.0001$) and stroke volume (SV) (data not shown) but did not affect mean pulmonary artery pressure, pulmonary arterial wedge pressure, or heart rate (Figure 1). Contrary to previous systemic studies (11,13), (Pyr[1])apelin-13 did not cause a significant reduction in SVR (Figure 1).

EXERCISE. During exercise (n = 9), there were no differences in any of the hemodynamic variables except PVR (Figure 2) and heart rate. The increase in PVR was attenuated during (Pyr[1])apelin-13 infusion ($p < 0.05$), and this was associated with an increase in heart rate ($p < 0.01$).

EFFECT OF CONCOMITANT PDE5 INHIBITORS. On post hoc analysis, there was a more marked reduction in PVR (reduction by 1.4 WU versus 0.4 WU) ($p < 0.0001$) and increase in CO (increase by 0.6 l/min vs. 0.35 l/min) ($p < 0.0001$) (Figure 3) with (Pyr[1])apelin-13 when compared with saline placebo in patients who were already established on treatment with phosphodiesterase-5 (PDE5) inhibitors (n = 11). This group also showed a reduction in SVR (data not shown; p = 0.0009). There was no change in these variables for patients who were not established on PDE5 inhibitors (Figure 3).

DISCUSSION

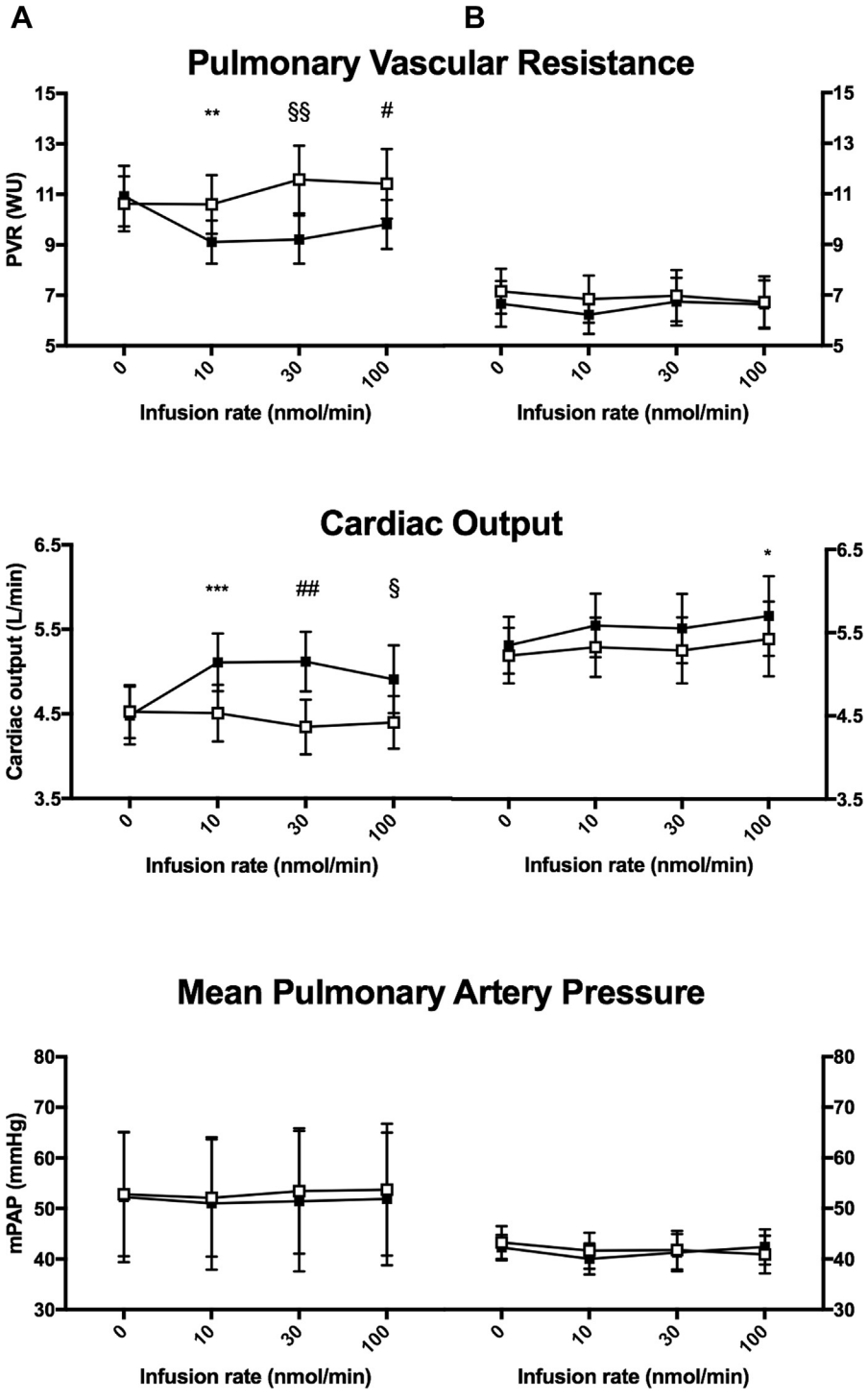
In patients with PAH, we have demonstrated for the first time that short-term intravenous (Pyr[1]) apelin-13 infusion associated with improvements in pulmonary hemodynamics, including a fall in PVR and an increase in CO and stroke volume. This effect was seen even in those patients already on medication for pulmonary hypertension and was particularly prominent in patients maintained on concomitant PDE 5 inhibitor therapy, consistent with an effect on cyclic GMP. We believe that apelin agonism is a potentially important and novel therapeutic target for intervention in patients with PAH.

The current recommendations for treatment goals as set out at the fifth World Symposium on Pulmonary Hypertension advise normalization of right ventricular function which is defined as right atrial pressure <8 mmHg and cardiac index of >2.5 l/min/m^2 (22). We have demonstrated that intravenous (Pyr[1]) apelin-13 infusion was able to improve CO and reduce PVR in patients with PAH. This suggests that, in addition to standard therapy, apelin agonism may have a potential role in helping these patients achieve their therapeutic targets.

This study was designed as an invasive study because this is the most accurate method of determining changes in hemodynamic variables. The study design was a within-subject, blinded, randomized, crossover study in order to enhance statistical power and robust comparisons of effects. We designed the study to measure both resting and exercise hemodynamics because patients with PAH are more symptomatic during activity, and we hoped to demonstrate that apelin has therapeutic benefit both at rest and during exercise. Interestingly, we were able to demonstrate improvements in pulmonary hemodynamics at rest, but surprisingly, improvements were not greater when the subjects exercised. This may be because of the large changes in hemodynamics caused by exercise, which may have obscured the more subtle changes observed at rest. It may be that longer-term apelin agonism could lead to an improvement in exercise pulmonary hemodynamics, and this may be worth examining in future studies.

The apelin system may have major relevance for patients with PAH. Dysfunction of bone morphogenetic protein (BMP) signaling is an important pathway in the pathogenesis of PAH (23), and it appears to influence apelin production as a potential mediator of

FIGURE 3 **Patients With or Without PDE5 Inhibitor Treatment**



Hemodynamic changes during infusion of (Pyr[1])apelin-13 **(solid squares)** or matched saline placebo **(open squares)** in patients with pulmonary arterial hypertension who are **(A)** on treatment with PDE5 inhibitors or **(B)** not on treatment with PDE5 inhibitors. Data are reported as mean ± SEM. *p = 0.049, **p = 0.0127, *** p = 0.0042, §p = 0.0039, §§p = 0.0017, #p = 0.0011, ##p = 0.0003, 2-way analysis of variance with post hoc Bonferroni tests. Abbreviations as in Figure 1.

the pathogenesis of PAH. In a long-term hypoxic rodent model of right ventricular failure (24), apelin had inotropic effects, and treating rats with monocrotaline-induced pulmonary hypertension with an apelin infusion led to improved right ventricular mass and hemodynamic variables (14). In human studies, apelin expression is reduced in the pulmonary artery endothelial cells (PAECs) of patients with idiopathic pulmonary arterial hypertension (15). Reducing apelin in human PAEC by siRNA impairs PAEC survival and promotes pulmonary artery smooth muscle cell proliferation in man (15). Furthermore, apelin can directly suppress vascular smooth muscle cell proliferation in response to growth factors, and is proapoptotic (15). Importantly, long-term apelin administration was associated with positive inotropism without any evidence of myocardial hypertrophy in murine models of PAH (6).

In previous studies, we provided the first evidence that apelin has vasoactive actions in humans. We showed that apelin causes vasodilatation in vivo in the human forearm circulation of healthy volunteers through a predominantly NO-dependent mechanisms, and that this effect is preserved in patients with heart failure (11,13). Furthermore, we showed that apelin is a direct coronary vasodilator and increases myocardial contractility in humans. It caused a reduction in both peak and end-diastolic left ventricular pressures (13). Here, we show that similar effects are also seen in pulmonary hemodynamics in patents with PAH.

It is also noted that there is not a dose-effect relationship, with a plateauing of PVR and CO at the higher infusion rates (**Figure 1**). This is consistent with results seen in our previous studies (13) and is why we had chosen to reduce the doses of our infusion rates during this study. This may reflect the doses we have chosen or an "all or nothing" effect.

We included patients who were taking PDE5 inhibitors in this study. It is known that in ex vivo myography studies, inhibition of NO attenuated apelin-induced vasorelaxation in human mesenteric arteries (5). As we demonstrated in our previous clinical study (11), the effects of apelin are partially attenuated with an "NO clamp" (concomitant balanced infusion of a NO donor and a NO synthase inhibitor) suggesting that apelin causes arterial vasodilation via a NO-dependent manner. PDE5 inhibitors also act on the NO pathway by preventing degradation of cGMP. We felt it was important to include this group for recruitment in our study to determine whether there was any synergistic vasodilatory effect when combining 2 drugs that act on the NO-cGMP pathway. Interestingly, on post hoc analysis, the group of patients established on PDE5 inhibitors had more marked improvements in PVR, CO, and SV, whereas the group who were not on treatment with PDE5 inhibitors (includes treatment naive and those on treatments other than PDE5 inhibitors) showed no demonstrable effect. This study was not powered to investigate this effect directly, but it would be important for future studies to explore this interaction further. It should also be noted that the group on PDE5 inhibitors had more severe baseline hemodynamic abnormalities, and demonstrated a reduction in SVR with apelin infusion consistent with our previous observations in patients with heart failure (11,13). It was not possible to assess the symptomatic impact of these short-term changes in the patients as they were supine on the catheterization table.

Currently, there is a great deal of interest in upfront combination treatment for PAH to determine whether this has long-term beneficial effects in patients with PAH by the establishment of early control and preservation of RV function. These benefits need to be balanced with the potential for drug-drug interactions and potential adverse effects. We did not demonstrate any adverse effects in our study, but this would need to be explored further in long-term studies.

This study examined the short-term effects of apelin administration, and longer studies are needed to determine long-term hemodynamic benefits, as well as potential long-term adverse effects. We have previously determined the effect of prolonged systemic infusion of apelin in man. During 6-h infusions of apelin in healthy volunteers and patients with heart failure, apelin was well tolerated and its inotropic actions were maintained, with a sustained increase in cardiac index and left ventricular ejection fraction (12). Furthermore, we previously monitored pulmonary artery blood flow during the first hour of apelin infusion using echocardiography and found this to be increased with apelin infusion versus saline placebo. This suggests that apelin will have sustained benefits on pulmonary vascular hemodynamics.

Apelin has also been shown in preclinical studies to target pulmonary vascular remodeling (15), so not only does it have beneficial hemodynamic effects in the human pulmonary vasculature, but it may also prevent the pulmonary vasculature remodeling seen in PAH. However, this has yet to be established.

(Pyr^{1})apelin-13 peptide is unlikely to represent a viable long-term therapeutic intervention for PAH

because it would require continuous intravenous systemic infusion. However, our study has demonstrated that activation of the apelin-APJ pathway led to an important improvement in PVR and CO even in patients established on current PAH therapies. To make apelin agonism a viable potential treatment for patients with PAH, further drug development is required to develop an oral or inhaled preparation that can be easily delivered to enable prolonged apelin agonism, in order to avoid the difficulties of long-term infusion treatments well demonstrated with prostacyclin. Development of orally active drugs that can cause long-term apelin agonism would be an exciting approach to the treatment of PAH. There are already synthetic agonists of the apelin receptor showing some promise in research (25).

CONCLUSIONS

We have shown here that short-term apelin infusion reduces PVR and increase CO and stroke volume in patients with PAH. Due to its short-term hemodynamic effects, the apelin pathway may be an exciting potential therapeutic target for the treatment of PAH. Further studies are required to determine the long-term effects of APJ agonism in PAH, and development of an oral or inhaled preparation that can easily deliver prolonged apelin agonism is urgently needed.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: This study has shown that APJ agonism reduces PVR and increases CO in patients with PAH in the short-term setting. Post hoc analysis suggests that these hemodynamic changes may be enhanced in those patients on concomitant phosphodiesterase-5 inhibitors.

TRANSLATIONAL OUTLOOK: Further research is required to look at the long-term effects of APJ agonism on patients with PAH. Development of an oral preparation that can cause prolonged APJ agonism will be necessary to allow further research into the effects of long-term APJ agonism and to consider APJ agonism as a potential treatment for patients with PAH.

REFERENCES

1. Morrell NW, Adnot S, Archer SL, et al. Cellular and molecular basis of pulmonary arterial hypertension. J Am Coll Cardiol 2009;54:S20–31.

2. NHS Digital. Fifth Annual Report: Key Findings From the National Audit of Pulmonary Hypertension of the United Kingdom, Channel Islands, Gibraltar and Isle of Man. Leeds, UK: Health and Social Care Information Centre, 2015:1–38.

3. Tatemoto K, Hosoya M, Habata Y, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 1998;251:471–6.

4. Kleinz MJ, Skepper JN, Davenport AP. Immunocytochemical localisation of the apelin receptor, APJ, to human cardiomyocytes, vascular smooth muscle and endothelial cells. Regul Pept 2005;126:233–40.

5. Salcedo A, Garijo J, Monge L, et al. Apelin effects in human splanchnic arteries. Role of nitric oxide and prostanoids. Regul Pept 2007;144:50–5.

6. Ashley E, Powers J, Chen M, et al. The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading in vivo. Cardiovas Res 2005;65:73–82.

7. Lee DK, Cheng R, Nguyen T, et al. Characterization of apelin, the ligand for the APJ receptor. J Neurochem 2000;74:34–41.

8. Tatemoto K, Takayama K, Zou MX, et al. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. Regul Pept 2001;99:87–92.

9. Cheng X, Cheng XS, Pang CCY. Venous dilator effect of apelin, an endogenous peptide ligand for the orphan APJ receptor, in conscious rats. Eur J Pharmacol 2003;470:171–5.

10. Berry MF, Pirolli TJ, Jayasankar V, et al. Apelin has in vivo inotropic effects on normal and failing hearts. Circulation 2004;110 Suppl 1:II187–93.

11. Japp AG, Cruden NL, Amer DAB, et al. Vascular effects of apelin in vivo in man. J Am Coll Cardiol 2008;52:908–13.

12. Barnes GD, Alam S, Carter G, et al. Sustained cardiovascular actions of APJ agonism during renin-angiotensin system activation and in patients with heart failure. Circ Heart Fail 2013;6:482–91.

13. Japp AG, Cruden NL, Barnes G, et al. Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure. Circulation 2010;121:1818–27.

14. Falcao-Pires I, Goncalves N, Henriques-Coelho T, Moreira-Goncalves D, Roncon-Albuquerque R, Leite-Moreira AF. Apelin decreases myocardial injury and improves right ventricular function in monocrotaline-induced pulmonary hypertension. Am J Physiol Heart Circ Physiol 2009;296:H2007–14.

15. Alastalo TP, Li M, de Jesus Perez V, et al. Disruption of PPARγ/β-catenin-mediated regulation of apelin impairs BMP-induced mouse and human pulmonary arterial EC survival. J Clin Invest 2011;121:3735–46.

16. Jing ZC, Jiang X, Han Z-Y, et al. Iloprost for pulmonary vasodilator testing in idiopathic pulmonary arterial hypertension. Eur Respir J 2009;33:1354–60.

17. Sulica R, Dinh HV, Dunsky K, Fuster V, Poon M. The acute hemodynamic effect of IV nitroglycerin and dipyridamole in patients with pulmonary arterial hypertension: comparison with IV epoprostenol. Congest Heart Fail 2005;11:139–44;quiz 145–6.

18. Petkov V, Ziesche R, Mosgoeller W, et al. Aerosolised iloprost improves pulmonary haemodynamics in patients with primary pulmonary hypertension receiving continuous epoprostenol treatment. Thorax 2001;56:734–6.

19. Apostolopoulou SC, Rammos S, Kyriakides ZS, et al. Acute endothelin A receptor antagonism improves pulmonary and systemic haemodynamics in patients with pulmonary arterial hypertension that is primary or autoimmune and related to congenital heart disease. Heart 2003;89:1221–6.

20. Grimminger F, Weimann G, Frey R, et al. First acute haemodynamic study of soluble guanylate cyclase stimulator riociguat in pulmonary hypertension. Eur Respir J 2009;33:785–92.

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21. Voswinckel R, Reichenberger F, Gall H, et al. Metered dose inhaler delivery of treprostinil for the treatment of pulmonary hypertension. Pulm Pharmacol Ther 2009;22:50–6.

22. McLaughlin VV, Gaine SP, Howard LS, et al. Treatment goals of pulmonary hypertension. J Am Coll Cardiol 2013;62 Suppl:D73–81.

23. Machado RD, Eickelberg O, Elliott CG, et al. Genetics and genomics of pulmonary arterial hypertension. J Am Coll Cardiol 2009;54:S32–42.

24. Dai T, Ramirez-Correa G, Gao WD. Apelin increases contractility in failing cardiac muscle. Eur J Pharmacol 2006;553:222–8.

25. Brame AL, Maguire JJ, Yang P, et al. Design, characterization, and first-in-human study of the vascular actions of a novel biased apelin receptor agonist. Hypertension 2015;65:834–40.

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